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Comparison Of The Widal Test With Salmonella Typhi Isolation From Typhoid Fever Patients In Jakarta Indonesia*

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Introduction

The Widal test has gained universal but controversial acceptance as an aid to diagnose typhoid fever in lieu of *Salmonella typhi* isolation. The Widal test, however, is neither sensitive nor specific since *S. typhi* O antigen cross reacts with antibodies produced by other *Salmonella* that contain the same antigenic determinants as *S. typhi*. This test defect can lead to an erroneous diagnosis of typhoid fever. In addition, the determination of a significant Widal titer is difficult because of individual variation depending upon prior exposure and whether a person lived in a low prevalence, endemic or epidemic typhoid fever area. Finally, a significant number of persons infected with *S. typhi* fail to produce detectable antibodies and would therefore be diagnosed as not having typhoid fever based on Widal results alone.

With these various limitations in mind, we decided to report the results from our experience in Jakarta, Indonesia where we compared the Widal and culture results. The results may provide additional information that will make the serologic interpretation more meaningful for this geographic area.

Materials and methods

Patients. The study group included a total of 197 hospitalized patients in R.S. Karantina (Infectious Diseases Hospital), Jakarta. For inclusion in the study a patient had a history of fever of at least one week duration, fever at admission and at least 2 of the following: abdominal pain, mental confusion, constipation, hepatomegaly or splenomegaly. Healthy control subjects included 13 laboratory and 33 hospital employees.

Clinical Specimens. Upon hospital admission, blood was drawn, 4 rectal swabs obtained and a urine specimen collected. Thereafter, blood was drawn daily until the patient was afebrile. Rectal swabs were obtained at weekly intervals and upon discharge and urine specimens were collected daily until the patient was discharged.

The blood (3 ml) was added to 15 ml of 10% Oxgall (8) (Oxoid Company, England). Two rectal swabs were placed into Amies transport medium (Difco Laboratories, Detroit, Michigan) and 1 each into 3 ml each of mannitol selenite (MSB) (7) and dulcitol selenite (DSB) (11) *Salmonella* enrichment broths (Oxoid Company, England). The specimens were then transported to the laboratory within 1 hr after collection, the blood culture and MSB and DSB incubated 18-24 hr at 37C and the urine centrifuged at 3000 x g and the sediment added to MSB. One swab from the Amies transport medium was put into MSB and the second swab was used to inoculate MacConkey (MAC), desoxycholate citrate lactose sucrose (DCLS) and *Sal-*

monella Shigella (SS) agar media (Difco Laboratories, Detroit, Michigan). This swab was then put into DSB. All cultures were incubated 18-24 hr at 37C. Following incubation, all MSB and DSB enrichment cultures were subcultured to MAC, DCLS and SS agar plates. Blood cultures were subcultured daily, through 8 days or until positive for *Salmonella* to MAC, DCLS, and SS agar plates.

As many *Salmonella*-like colonies as were available to a maximum of 10 from each medium were subcultured to Kligler Iron (KIA), lysine iron, motility indole ornithine, lysine decarboxylase and urea agar media (Difco Laboratories, Detroit, Michigan). Growth was taken from the KIA slant, in the 5 tube screen that gave a presumptive *Salmonella* profile and used to determine the serological reaction (4).

Widal Serology. The Widal slide agglutination method was used to determine Salmonella, O, H, A and B antibody levels (2). Commercial antigens were used (Difco). Acute and convalescent specimens were obtained at least 2 weeks apart. The acute serum sample was obtained when the blood was drawn for the initial blood culture.

Results

S. typhi – Widal O titers: Results in Table 1, show that sera from 62% of the bacteriologically confirmed typhoid fever patients had Widal O titer $\geq$ 40. Increasing the significant titer limits correspondingly decreased the diagnostic value of the Widal test until at a titer of $\geq$ 320 only 20% of the patients developed a significant titer. The Widal titer of $\geq$ 40 diagnostic for 36% of those patients from whom *S. typhi* was not isolated and the value again decreased as the titer limit was increased. Twenty eight percent of the confirmed and 20% of the unconfirmed typhoid fever patients developed a 4-foll O antibody increase. The percentage may have been higher but determining a 4-fold increase with an acute titer of $\geq$ 160 was not possible because we did not test past a dilution of 320. The number of patients demonstrating a 4-fold O titer increase, however, could not have exceeded 53% even if all those patients with acute titers of $\geq$ 160 were considered to have had 4-fold O titer increases.

S. typhi – Widal A and B titers: The Widal *S. paratyphi* A and B antibody titers in sera from the suspected typhoid fever patients are also shown in Table 1. Twelve percent (17) of the confirmed and 10% (5) of the unconfirmed patients developed an acute A titer of $\geq$ 40 while 4% (6) of confirmed patients developed a 4-fold titer increase. A similar pattern was shown for *S. paratyphi* B antibody levels with 16% (23) of the confirmed and 14% (7) of the unconfirmed patients having acute titers of $\geq$ 40 and 6% (3) of the unconfirmed patients, a 4-fold titer difference.

Non-S. typhi Widal O, A and B titers: Seven *S. paratyphi* A and 7 *S. oranienburg* were the only other enteric pathogens isolated from 14 separate patients in this study. Three patients with *S. paratyphi* A infections had an O antibody titer of 40, 7 an A titer of 80 and 1 a B titer of 40. There was a 4-fold O antibody increase in the sera from 1 patient with *S. paratyphi* A infection while a 4-fold A antibody increase was noted in sera from 2 patients with *S. paratyphi* A infections. None of the sera from the 7 patients with *S. oranienburg* infection cross reacted with *Salmonella* A or B antigens but acute and convalescent sera from three of these patients contained 4-fold O antibody increases.

Discussion

Varying opinions have been expressed about the efficacy of using the Widal agglutination test as a diagnostic aid for typhoid fever and what constitutes a significant *Salmonella* group O antibody titer. *Wicks, et al,* (14) in Africa had to use a reciprocal titer of $\geq$ 480 to accurately diagnoses 60% of those patients with bacteriologically confirmed typhoid fever. This titer was found suitable independent of the length of pyrexia and was used because of apparent high antibody levels developed from an anamnestic reaction during constant exposure of their patients in an endemic typhoid fever area. They also found that Widal titers in 1076 other consecutive pyrexia patients were only diagnostic for 9% of the *S. typhi* infections whereas 6% were falsely positive and 77% were completely negative.

Gulati, et al, (5) in India studied 98 patients with suspected typhoid fever. Only 46/98 (47%) had positive blood cultures whereas all 98 patients had Widal O titers of $\geq$ 200. Most patients (58%) developed the titer by 1 week after onset of symptoms, 28% by the second week and the remainder by the third week. They also found that the convalescent serum titer from 39 other bacteriologically proven typhoid fever patients rose in 17, remained the same in 12 and fell in 10. The convalescent values were obtained after chloramphenicol therapy. Corticosteroids administered simultaneously with some chloramphenicol dosages were considered to alter the immune response in 11 patients with equal acute and convalescent or falling convalescent serum titers.

Levine, et al. (9) studied one population of healthy persons living in an endemic area in Peru, one population of Mexican typhoid fever patients, one population of adult United States volunteers who developed acute typhoid while serving as controls as experimental challenges to evaluate typhoid vaccine and one population from Baltimore, Maryland, U.S.A. in an area not endemic for typhoid fever. They found that approximately 24% and 15% of all healthy Peruvians had O titer of $\geq$20 and $\geq$40, respectively. The O titer was most prevalent in the 15-19 year old age group. The endemic nature of the Peruvian area was considered the source of constant subclinical infection and concomitant immune response. Conversely, the prevalence of *Salmonella* O antibody titers of the healthy population from the non endemic area in Baltimore, Maryland was low. Only 2% of those had a $\geq$ 40 *Salmonella* O titer. Nearly, 70% and 80% of the volunteers who ingested *S. typhi* and developed acute typhoid fever had O titers of $\geq$ 40 after 1 and 2 weeks of illness, respectively. It was concluded that an

Table 1. Widal O, A and B titers of sera from 186 patients with clinical or bacteriologically confirmed typhoid fever.

S. typhi from blood and/or feces	*Widal O titer* 320	160	80	40	20	0	*Acute-convalescent 4-fold increase*	*Total*
+	27 (20)[1]	8 (3)	30 (22)	19 (14)	17 (12)	36 (16)	38 (28)[2]	137
–	5 (10)	2 (4)	3 (6)	8 (16)	11 (22)	20 (41)	10 (20)	49
	Widal A titer							
+	0 (0)	1 (1)	7 (5)	9 (6)	12 (9)	108 (79)	6 (4)	137
–	0 (0)	0 (0)	0 (0)	5 (10)	4 (8)	40 (82)	0 (0)	49
	Widal B titer							
+	2 (1)	0 (0)	8 (6)	13 (9)	13 (9)	101 (74)	0 (0)	137
–	0 (0)	2 (4)	0 (0)	5 (10)	5 (10)	37 (76)	3 (6)	49

1. Number of acute sera with titer (% of sera with titer).
2. Number of acute convalescent sera with 4 fold increase (% of acute convalescent sera with 4-fold increase.

O antibody titer of ≥ 40 was diagnostic of acute typhoid fever when related to persons from a non-endemic typhoid area. Most (93%) of the Mexican typhoid patients had O titers of ≥ 40 at hospital admission. The duration of illness was 15-18 days before the acute specimen was obtained. No baseline O titer levels were determined for the presence of O antibody in the healthy population.

Anderson, et al. (1) found that 70% of patients in Jakarta, Indonesia with *Salmonella* bacteremia had O antibody. Only 15% had a 4-fold antibody rise between the acute and convalescent specimes. Among the bacteremic patients, 44% had ≥ 160 O titers, 9% ≥ 160 *paratyphoid* A titers and 22% ≥160 *paratyphoid* B titers. Although there was an association between the specific *Salmonella* infectious agent isolated and the group specific antibody, cross reactions occurred and reduced the accuracy of the diagnosis based on Widal alone.

Gupta and *Rao* (6) found that of 26 bacteremic typhoid fever patients, 1 (4%) had a Widal O titer ≥ 320 in the acute phase specimen collected 2-5 days after onset of symptoms. The O titer in convalescent sera collected 9-12 days post-onset was ≥ 320 in 18 (69%) patients. *Nourmand* and *Mohsen* (6) showed that 138 (84%) patients with clinically suspected typhoid and paratyphoid fever had rising O titers of ≥ 160. From this population, 61 patients had culture proven bacteremia with *S. typhi,* 31 with *S. paratyphi* A and 32 with *S. paratyphi* B.

Santiago (12) compared the clinical diagnoses of 148 typhoid fever patients with the Widal test and culture results. She used a Widal O titer of ≥ 320 for a single acute specimen or a rise in O titer between the acute and convalescent specimens as diagnostic. Using these serological limits, the clinical interpretation and culture results, she concluded that only 30% of the patients had been bacteremic while 82% were Widal positive.

It becomes apparent from reading the literature that a "significant" Widal titer depends on the investigator doing the study. The range of diagnostic titers for *Salmonella* O antibody has been reported to between 40 and > 480. Not only is the interpretable range wide, a significant number of *S. typhi* bacteremic patients never elicit an immune response either because of therapy or a compromised immune system. Additionally, some patients with *S. paratyphi* A or B infections develop O and H antibodies that suggest typhoid fever. This is understood because *S. typhi,* 59 other group D *Salmonella,* and the 2 *S. paratyphi* species all contain factor 12 in their somatic O antigen (3). This cross reactivity prevents an absolute assessment of the exact etiologic agent even when the O titer is significantly elevated. There is no unanimity as to the significance of a *Salmonella* H antibody titer and most investigators agree it is almost meaningless for diagnosis.

The results of this study likewise showed that the Widal test was neither easy to interpret nor very sensitive since only 62% of those patients with bacteriologically confirmed typhoid fever had acute O titers of ≥ 40. With higher titer limits of ≥ 80 the sensitivity decreased significantly. Likewise, using a 4-fold antibody change as the diagnostic criterion would have limited the confirmed diagnosis to 28% if only the 4-fold increase titers were used and to approximately 50% if the ≥ 160 titers were added as assumed 4-fold increases.

Based on a significant *Salmonella* O antibody titer of ≥ 40 or a 4-fold increase between acute and convalescent sera, our results from 186 patients showed that 54% and 26%, respectively, of the patients had typhoid fever by Widal serology alone, in sharp contrast to the 74% of patients confirmed by bacteriology. However, the diagnosis was made for 36% of those patients from whom *S. typhi* was not isolated when using a ≥ 40 acute titer and 20% when using a 4-fold increase as the criteria. Thus it would appear the Widal was diagnostic of typhoid fever in about one-third of those instances at the most when the bacterium was not isolated by culture.

Cross reactions between Widal *Salmonella paratyphi* A and B antigens and *Salmonella* O antibody were present. Based on positive culture results alone, 40/137 (29%) of those patients with *S. typhi* infections had *S. paratyphi* A and B Widal titers ≥ 40 and 6/137 (4%) of the patients 4-fold rises against the same antigens. This would confuse an exact diagnosis of typhoid because in all instances the patients also had significant O titers of ≥ 80. Considering that factor 12 is common to *Salmonella* groups A, B and D, it is well known that cross reactions occur and antibody can be produced against any of those groups but leading to the impression that *S. typhi* was the etiologic agent. The sera from the 3 patients with *Salmonella* C_1 infection that had 4-fold O antibody increases were probably diagnostic for typhoid fever since they did not cross react with *S. paratyphi* A or B antigens and group C_1 does not have factors common to either *Salmonella* group A, B or D.

It would appear then, that using an acute titer of ≥ 40 is generally acceptable in Jakarta, Indonesia even though about 40% of those patients with bacteriologically documented typhoid fever would not be diagnosed solely by the Widal serology. Since most of the control subjects did not have a demonstrable Widal O titer, there might be a tendency to consider that the bacground level of O antibody among healthy persons in Jakarta was low even though most were probably exposed endemically. Actually, one would suppose that at least some of the hospital employees in constant contact with typhoid fever patients would have had detectable O antibody. The finding that only one had and O titer of 20 was interesting. If a much larger sample of the healthy population showed the same trend then a significant acute Widal O titer might be defined as one of ≥ 20 instead of ≥ 40.

The results of this study showed that in an Indonesian setting a significant Widal titer was not well defined, the test was not very sensitive or specific, it was diagnostic with certain restrictions, and that the diagnosis of typhoid fever still rested primarily on the isolation of *S. typhi* from the patient. *Schroeder* (3) wrote "serological tests for typhoid fever are nonspecific, poorly standardized, often confusing, and difficult to interpret. If serologic tests are used to diagnose typhoid fever, the titer for O antigen is the only meaningful value, and even this may be suppressed by early treatment with antibiotics, or elevated by immunization."

Ringkasan

Kadar antibodiserum Salmonella O, A dan B pada 186 penderita yang diduga menderita demam tifoid, telah diukur melalui test Widal. Hasilnya kemudian dibandingkan dengan hasil biakan *Salmonella typhi* yang diperoleh dari darah dan tinja penderita yang sama.

Hanya 84/137 (61%) dari mereka yang secara bakteriologik positip, yang mempunyai titer antibodi Salmonella $O > 40$. Sedangkan kuman *S. typhi* dapat diisolsikan dari 137/186 (74%) penderita.

Peningkatan sampai empat kali lipat kadar antibodi O selama antara masa akut dan konvalesen terdapat pada kira-kira 28% dari yang bakteriologik positip dan juga dari seluruh 186 penderita. Suatu diagnosis yang dianggap pasti terdapat pada 57% dari semua penderita yang hasil biakannya negatif, jika berpedoman pada titer widal O yang > 40 dan peningkatan titer sampai empat kali lipat antara masa akut dan konvalesen.

Reaksi silang pada serum akut yang > 40 antara antigen *S. paratyhis* A dan B dengan antibodi *S. typhi* O terdapat pada 36/137 (26%) kasus. Dan peningkatan titer empat kali lipat terdapat pada 6/137 (4%) kasus.

Nilai diagnostik dari test Widal menurun tajam jika batas titer yang signifikan melebar. Penelitian ini menunjukkan bahwa test Widal di Indonesia tidaklah sensitif ataupun spesifik dan harus digunakan secara hati-hati jika mendiagnosis demam tifoid.

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Based on a significant Salmonella O antibody titer of 40 or a 4-fold increases between acute and convalescent sera, results from 186

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